# *In vivo* evaluation of antihyperlipidemic, antihyperglycemic and hepatoprotective effects of *Vernonia anthelmintica* seeds in diet model

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**Abstract**: Anti-hyperglycemic, anti-hyperlipidemic and hepatoprotective effects of Vernonia anthelmintica seeds were evaluated in diet rat model. The study included forty eight Sprague Dawley rats of either sex with eight animals in each group. Except for control the disease control, standard and treatment groups were treated with high-fat high-sugar diet for 8 weeks. After confirmation of hyperlipidemia and hyperglycemia, the standard group received atorvastatin (2.1 $mg.kg^{-1}$), treatment groups received hexane extract, ethanol extract and water decoction of *Vernonia anthelmintica* seeds (300$mg.kg^{-1}$) for next 30 days. Disease control and control were given normal saline in volume equivalent to other groups. High-fat high-sugar diet was continued in all groups except control for 30 day period. Lipid profile, liver function tests and fasting blood sugar were analyzed in fasting blood samples. Cardiac risk parameters were calculated and results were analyzed by one way ANOVA using SPSS. All three tested extracts showed significant decrease in fasting blood glucose, LDL-C, triglycerides, VLDL-C and cardiac risk parameters as compared to disease control. However, HDL-C and cholesterol levels in treatment groups were found to be significantly increased compared to disease control. Furthermore, treatment groups showed significantly decreased AST and ALP levels compared to disease control. Hexane extract, ethanol extracts and water decoction of *Vernonia anthelmintica* seeds exhibited potential anti-hyperglycemic, anti-hyperlipidemic effects with favorable hepatic profile. However, further studies should be designed to strengthen these findings on mechanistic ground.

**Keywords**: Atherogenic index, anti-hyperglycemic, anti-hyperlipidemic, cardiac risk parameters, *Vernonia anthelmintica.*

## INTRODUCTION

Hyperlipidemia, or more accurately, dyslipidemia is known to be the greatest threat that contributes to the occurrence and aggravation of coronary heart disease (Neil *et al*., 1990) The primary causes of death worldwide are CHD, stroke, hyperlipidemia and atherosclerosis (Smith *et al*., 1993). Hyperlipidemia is defined as elevated levels of all lipids and lipoproteins (total cholesterol (TC), low density lipoprotein (LDL-C) and very low density lipoproteins (VLDL-C). The only exception is high density lipoproteins (HDL-C) that are supposed to be decreased. Among these, the most common etiologies of ischemic heart disease are hypercholesterolemia and hypertriglyceridemia (Jackson and Beaglehole, 1995).

Apart from medication, diet plays an important role in controlling lipid levels in blood. Various studies claimed that uncontrolled use of high fat diet is the reason behind insulin resistance that results in diabetes mellitus, its associated complications and oxidative stress (Park *et al*., 2011).

Present study has undertaken high-fat high-sugar (HFHS) rat model of hyperlipidemia as it is found to be a very close animal model to human disease etiology. Utilization of trans and saturated fatty acids are the key players for the induction of insulin resistance. Vanaspati ghee contains trans and saturated fatty acids. Similarly, coconut oil also contains saturated fatty acids in high proportions (Munshi *et al*., 2014). High sugar utilization increases the body weight and decreases circulating leptin levels resulting in insulin resistance, increased serum insulin levels, hypertriglyceridemia and hence hyperglycemia (Elliott *et al*., 2002).

*Vernonia anthelmintica* belongs to *Astraceae* (*compositeae*) family. The most frequently used synonym is *Centratherum anthelminticum*. (Bewley *et al*., 2006). This study elucidates the effects of hexane extract, ethanol extract and water decoction of *Vernonia anthelmintica* on hyperlipidemia. Furthermore, the effects on liver function and glucose metabolism have also been evaluated in order to establish safe use of these herbal drugs in treating hyperlipidemia.

## MATERIALS AND METHODS

### *Collection and identification of seeds*

The seeds of *Vernonia anthelmintica* were procured from herbal market, authenticated by growing in Center of Plant Conservation and identified by Prof. Dr. Anjum Parveen, Director, Plant Conservation Center, Department

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of Botany, University of Karachi against Herbarium number: G.H. No 92629.

***Preparation of extract***

The seeds were cleaned manually for removal of impurities like straws and dirt. The seeds were ground to coarse powder in a mechanical grinder and soaked in n-hexane for 10 days with occasional shaking. On the11$^{th}$ day the solvent containing hexane soluble constituents was filtered through muslin cloth. The filtrate was loaded in rotary evaporator for evaporation of solvent at 50$^{o}$C at high pressure to obtain yellowish green oily hexane extract of *Vernonia anthelmintica* referred to as HEVA (hexane extract of *Vernonia anthelmintica*). The residual after filtration was soaked again in ethanol to obtain brownish black gummy ethanol extract of *Vernonia anthelmintica* referred to as EEVA (ethanol extract of *Vernonia anthelmintica*) by the same methodology described for hexane extract. To prepare water decoction of *Vernonia anthelmintica* seeds (WDVA), its coarse powder was boiled in distilled water each time before dosing.

***Selection of animals***

Sprague-Dawley rats (n=48) weighing 250-350gm were brought from HEJ research institute of Chemistry, University of Karachi, Pakistan. Before starting any experimentation, all the animals were kept in the animal house of Department of Pharmacology, Faculty of Pharmacy under close monitoring in standard laboratory environment with temperature at 25°C, relative humidity at 60-70% and 12h light and dark cycle for at least one week. During this acclimatizing phase, all animals were monitored for their general health and gross behaviours including edema, diarrhoea, urination pattern, ulceration, skin and fur conditions and drowsiness or anxiety signs. Animal handling and sample collection were according to CLSI approved guidelines H21-A5 (Adcock, 2008).

***Induction of hyperlipidemia and hyperglycemia: (high-fat high-sugar model)***

After acclimatizing phase, the animals were numbered, weighed and randomly stratified into 6 groups of 8 animals each. Control group received standard laboratory diet whereas hyperlipidaemia and hyperglycaemia were induced in remaining 5 groups by adding high-fat high-sugar (HFHS) diet along with standard laboratory diet. Fat components of HFHS diet consisted of locally available Vanaspati ghee and coconut oil in the ratio of 3:1 (v/v) whereas sugar contents contained 25% fructose w/v in drinking water ad libitum (Munshi *et al*., 2014). HFHS diet was given in the dose of 3 ml/kg to all groups orally except control for twelve weeks.

***Experimental design***

The rats were randomly grouped into six groups. The first group served as normal control (NC). From the remaining hyperlipidemic and hyperglycaemic groups, one group received distilled water and was designated as disease control (Adcock, 2008); one group received standard antihyperlipidemic drug atorvastatin (2.1mg/kg) and was designated as Standard control (SC); and, the last three groups received HEVC, EEVC and WDVC (300 mg/kg) and designated as test groups (HEVC, EEVC and WDVC) respectively. Drug treatment and distilled water were given to all groups for the next 4 weeks.

***Sample collection***

At the end of the experimental period, 12 hr fasting blood sample of animals was drawn after decapitation. Serum was then assessed for fasting blood glucose, lipid profile (LDL-C, HDL-C, VLDL-C, Total cholesterol, triglycerides and Cholesterol/ HDL-C ratio) and liver function tests (ALT, AST, ALP, GGT and total bilirubin). Cardiac risk parameters including LDL-C/HDL-C ratio and Cholesterol/HDL-C ratio were calculated by simple mathematical formula whereas atherogenic index was calculated using following formulae:

$$\text{Atherogenic Index} = \frac{\text{Total cholesterol HDL - C}}{\text{HDL - C}}$$

(Schulpis and Karikas, 1998) eq: 1

***Biochemical analysis***

For biochemical analysis, 7ml of blood was collected in a yellow top serum collection tube and set to centrifugation at 3000 rpm for 10 minutes to separate serum from whole blood. This supernatant serum was aspirated using pipettes and stored in Eppendorf tubes for testing on Humalyzer 3000. It is an automated chemistry analyser used for biochemical analysis using standard kits provided by Human Germany.

***Lipid profile assessment***

LDL-C and Cholesterol were estimated by the CHOD-PAP method. However, triglycerides levels were assessed by GPO-PAP method (Trinder, 1969). HDL-C was assessed by the method of Friedewald et al (Friedewald *et al*., 1972).

***Fasting blood glucose assessment***

Fasting blood glucose was determined by GOD-PAP enzymatic colorimetric test method (Barham and Trinder, 1972).

***Assessment of liver function***

ALT (alanine aminotransferase) and AST (aspartate transferase) were analysed according to the recommendations of the expert panel of International Federation of Clinical Chemistry (IFCC). Kinetic method for the determination of ALAT without pyridoxalphosphate activation is used in this study (Schumann *et al*., 2002). GGT(Gamma glutamyl transferase) was analysed by colorimetric method

according to Persijin and Vanderslik that was standardized against recommended IFCC method (Persijn and van der Slik, 1976). ALP (Alkaline phosphatase) was analysed by optimized standard method according to the recommendations of German Clinical Chemistry Association using Diethanolamine buffer and DGKC orthophosphoric monoester phosphohydrolase (Keiding *et al.*, 1974). Bilirubin Direct and Total was analysed by a photometric test referred to as modified by Jendrassik/ Grof Method (Mori, 1978).

## STATISTICAL ANALYSIS

All values are expressed in terms of mean ± S.E.M (standard error to the mean) and compared using one way ANOVA by using SPSS version 20.0. Values with P <0.05 were taken as significant and with p<0.005 were considered as highly significant.

## RESULTS

### *Antihyperlipidemic activity*

Table 1 shows the antihyperlipidemic effects of HEVA, EEVA and WDVA as compared to normal control (NC), disease control (Adcock, 2008) and atorvastatin (SC) standard antihyperlipidemic drug.

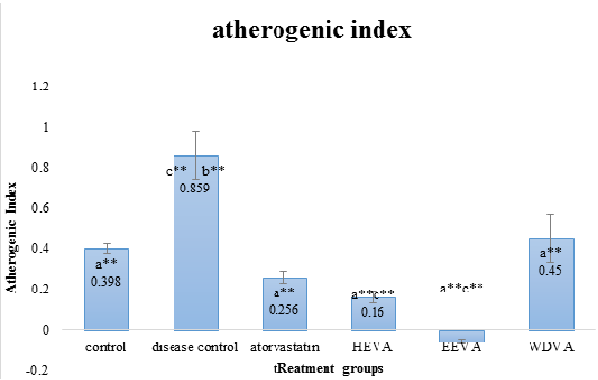



**Fig. 1**: Effects of Extracts of *Vernonia anthelmintica* on atherogenic index in HFHS rat model

In comparison to NC, DC shows significant difference in terms of serum cholesterol, triglycerides, LDL-C and VLDL-C. Standard drug atorvastatin showed no significant difference as compared to normal control whereas HEVA, EEVA and WDVA showed significantly higher HDL-C levels and the latter two are also significantly different in terms of total cholesterol as compared to control.

In comparison to DC, all NC and treatment groups are significantly different in terms of triglycerides, LDL-C and VLDL-C. In addition to these parameters, EEVA also shows significant increase in HDL-C levels.

In comparison to SC, DC shows significant difference in terms of cholesterol, triglycerides, LDL-C and VLDL-C. HEVA, EEVA and WDVA also show significant higher levels of HDL-C and due to this reason EEVA and WDVA show significant higher total cholesterol levels as compared to SC.

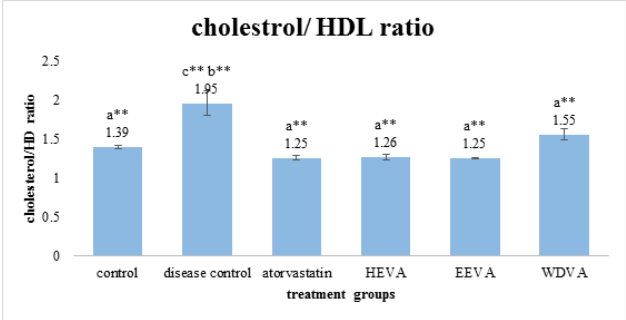
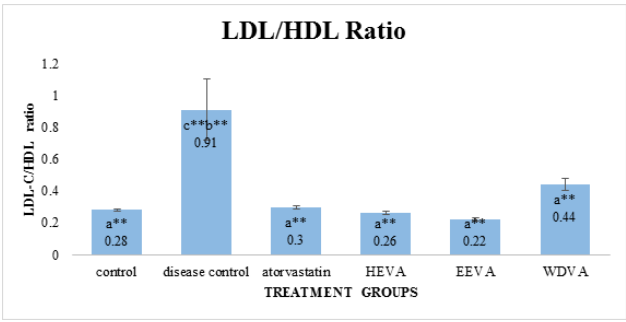



**Fig. 2**: Effects of extract of *Vernonia anthelmintica* on Cholesterol/HDL-C ratio in HFHS rat model




n=8
Values are expressed as mean ±S.E.M.
*P ≤ 0.05 significant; **P ≤0.005 highly significant
'a' significantly different as compared to disease control.
'b' significantly different as compared to standard.
'c' significantly different as compared to control.
HEVA= Hexane extract of *Vernonia anthelmintica.*
EEVA= Ethanol extract of *Vernonia anthelmintica.*
WDVA= Water decoction of *Vernonia anthelmintica.*
HFHS= high fat high sugar

**Fig. 3***:* Effects of Extracts of *Vernonia anthelmintica* on LDL-C/HDL-C ratio in HFHS rat model

### *Cardiac risk parameters*

Table 2 and fig. 1, fig. 2 and fig. 3 shows the cardiac risk parameters including atherogenic index, Cholesterol /HDL-C ratio and LDL-C/HDL-C ratio of HEVA, EEVA and WDVA as compared to normal control (NC), disease control (Adcock, 2008) and standard control (SC) atorvastatin. In comparison to NC, DC shows significant increase in terms of atherogenic index, cholesterol/HDL-C ratio and LDL-C/HDL-C. SC and test groups show no significant difference as compared to normal control. In comparison to DC, all NC, SC and test groups (HEVA, EEVA and WDVA) are significantly low in terms of atherogenic index, cholesterol/HDL-C ratio and LDL-C/HDL-C.

### *Anti-diabetic profile*

Table 3 shows the fasting blood glucose levels of animals receiving HEVA, EEVA and WDVA as compared to control (NC), disease control (Adcock,2008) and

atorvastatin (SC). In comparison to NC and SC, DC shows significant increase in fasting blood glucose levels whereas in comparison to disease control, all control and treatment groups (SC, HEVA, EEVA and WDVA) show significantly decreased blood glucose levels.

***Hepatic profile***

Table 4 shows the Liver function tests of HFHS rat model receiving HEVA, EEVA and WDVA as compared to control (NC), disease control (Adcock, 2008) and atorvastatin (SC). There is no significant difference in levels of GGT and ALT in different groups. Standard drug shows significantly lower ALP levels as compared to disease control. AST and TBR show different patterns of significant differences among different groups. WDVA shows significantly higher AST levels as compared to NC.

## DISCUSSION

Atherosclerosis progressing to cardiovascular disease (CVD) is the principal cause of morbidity and mortality worldwide. Atherosclerosis refers to a complex set of complications that involves many cellular components along with varying circulating mediators that lead to an inflammatory state. Lesion of atherosclerosis starts with the deposition of lipoproteins, macrophages derived from monocytes and lymphocytes along with arterial wall. Even with the advancement in cardiology medicine, atherosclerosis remains a prominent medical complication (Burnett, 2004).

Current pharmacotherapy of hyperlipidaemia has various side effects whereas herbal drugs are supposed to be effective in lowering lipids and hence CVD. Herbal medicines are cheap and easily available and have comparatively low risks (Kaur and Meena, 2013). In the present study, high-fat high-sugar hyperlipidemic rat model was used for the assessment of antihyperlipidemic effect of Vernonia anthelmintica extracts as it is very close to metabolic syndrome caused by high dietary consumption of fat and sugar presumably due to unhealthy diet practices in our population (Munshi et al., 2014).

Lipid profile, liver function tests and fasting blood glucose levels were estimated at the end of study period. The results revealed that in rats, LDL-C levels are far less compared to HDL-C levels, in all control and treated groups except for disease control. Although these results are contrary to human LDL-C levels which are greater than HDL-C levels, it is in accordance with various previous studies that also revealed same pattern of lipid levels in rats (Munshi *et al.,* 2014*,* Lehmann *et al.,* 1993).

High levels of low density lipoproteins (LDL-C) have long been recognized as a primary risk factor for the development of cardiovascular disease due to atherosclerosis and hence hold a principal target for the treatment and prevention of disease. In the present study, disease control group showed significantly higher levels of LDL-C cholesterol, while 30 days dosage of standard drug atorvastatin, HEVA, EEVA and WDVA significantly reduced LDL-C levels. The LDL-C lowering effect is more profound in atorvastatin followed by EEVA, HEVA and WDVA.

Increase in the levels of triglycerides has been reported as an independent risk contributor to CVD (Cullen, 2000). It is also reported that it might be a possibility that inspite of acting as sole atherogenic agents, high levels of triglycerides may serve in increment of triglyceride rich remnant lipoproteins that may lead to atherosclerosis (Havel, 1990). In the present study, elevated levels of triglycerides in disease model were significantly reduced by Vernonia anthelmintica extracts and standard drug. This decrease was of higher magnitude in EEVA followed by atorvastatin and HEVA whereas WDVA revealed non-significant alterations in triglycerides.

Several studies have indicated the role of flavonoids in cholesterol synthesis and shown to have both stimulatory and inhibitory effects depending on the type and dose of flavonoid (Gebhardt, 2001). Moving more towards mechanistic approach, flavonoids are known to produce their cholesterol lowering effects by inhibition of cholesterol synthesis, modulation of HMG-CoA reductase activity, increasing biliary secretion of cholesterol, decreasing production of Apo-B100 and increasing LDL-C receptor expression (Pal et al., 2003; Park et al., 2001). Few studies also revealed the role of flavonoid in inhibition of lipases and serum triglycerides levels in animal models (Kawaguchi et al., 1997).

Hence flavonoids in most of the studies revealed that their use is associated with low total cholesterol, high HDL-C cholesterol and high HDL-C/LDL-C ratio (Vinson and Dabbagh, 1998).

Previous studies have shown the cholesterol lowering effects of alkaloids derived from plants. The main mechanism involved in protection of alkaloids against atherosclerosis is its cholesterol lowering effects. Alkaloids have shown increased LDL-C receptor expression. Alkaloids also control atherosclerosis due to its anti-oxidant, anti-inflammatory actions as well as its involvement in inhibition of vascular cell proliferation and endothelial dysfunction. Alkaloids e.g. berberine has shown to have total cholesterol, LDL-C, and triglyceride lowering potential as well as capability of increasing HDL-C (Pirillo and Catapano, 2015).

An associated study has revealed the presence of flavonoids and alkaloids in HEVA and EEVA that might be responsible for its anti-hyperlipidemic effects as WDVA lacks alkaloids as revealed in phytochemical

**Table 1**: Effects of *Vernonia anthelmintica* on hyperlipidemia in HFHS rat model

| Groups | Diet | Dose mg/kg | Cholesterol | triglyceride | HDL-C | LDL-C | VLDL-C |
|---|---|---|---|---|---|---|---|
| | | | mg/dl | | | | |
| Control (NC) | Normal | Distilled water | 54.50±3.05 a** | 46.25 ±2.25 a** | 39±2.05 | 11±0.92 a** | 9.0±0.46 a** |
| Disease control (DC) | HFHS | Distilled water | 102.50±9.19 c** b** | 83.25± 7.36 c** b** | 53± 2.4 | 48.5± 10.7 c** b* | 16.75±1.49 c* b* |
| Atorvastatin (SC) | HFHS | 2.1 | 57.75± 2.41 c** | 34.50± 2.78 a** | 46.25±2.38 | 14±1.035 a** | 6.75±0.55 a** |
| HEVA | HFHS | 300 | 80.25± 7.41 c* | 40.75±0.86 a** | 63.75±5.97 c** b* | 16.7± 1.49 a** | 8.0±0.26 a** |
| EEVA | HFHS | 300 | 99.25±5.38 c** b** | 29.25± 1.91 a** c* | 79± 3.69 a** c** b** | 18.25±1.82 a** | 5.75±0.31 a** c* |
| WDVA | HFHS | 300 | 97.25±1.23 c** b** | 46.25± 2.44 ** | 63.5± 3.36 c** b* | 29± 1.36 | 9.25±0.67 a** |

**Table 2**: Effects of seed extracts of *Vernonia anthelmintica* on cardiac risk parameters

| Groups | Diet | Dose mg/kg | Atherogenic index | Cholesterol/HDL-C ratio | LDL-C/HDL-C ratio |
|---|---|---|---|---|---|
| Control (NC) | Normal | Distilled water | 0.398± 0.023 a** | 1.39±0.02 a** | 0.28±0.01 a** |
| Disease control (DC) | HFHS | Distilled water | 0.859±0.12 c**b* | 1.95±0.16 c**b* | 0.91±0.19 c**b* |
| Atorvastatin (SC) | HFHS | 2.1 | 0.256±0.33 a** | 1.25±0.03 a** | 0.30±0.01 a** |
| HEVA | HFHS | 300 | 0.16± 0.031 a** | 1.26±0.04 a** | 0.26±0.01 a** |
| EEVA | HFHS | 300 | -0.06± 0.01 a**c** | 1.25±0.01 a** | 0.22±0.01 a** |
| WDVA | HFHS | 300 | 0.45±0.12 a* | 1.55±0.07 a* | 0.47±0.04 a** |

n=8
Values are expressed as mean ±S.E.M
*P ≤ 0.05 significant; **P ≤0.005 highly significant
'a' different as compared to disease control
'b' different as compared to standard
'c' different as compared to control
HEVA= Hexane extract of *Vernonia anthelmintica.*
EEVA= Ethanol extract of *Vernonia anthelmintica*
WDVA= Water decoction of *Vernonia anthelmintica*
HFHS= high fat high sugar

evaluation (Jamil *et al*., 2016). Hence its antihyperlipidemic potential is of lesser magnitude as compared to EEVA and HEVA which contains both flavonoids and alkaloids.

As total cholesterol is the sum of all cholesterols, EEVA, HEVA and WDVA do not show significant reduction in their levels as these test drugs increase HDL-C in the course of reducing LDL-C. Hence increase in cholesterol in disease control depicts the increment in LDL-C and triglyceride fraction whereas increase in total cholesterol in HEVA, EEVA and WDVA represent favourable increase in HDL-C.

Present study estimated the cardiac risk by calculating atherogenic index, cholesterol/ HDL-C ratio and LDL-C/HDL-C ratio with the help of measured lipid and cholesterol value. Disease control revealed significantly higher atherogenic index, cholesterol/ HDL-C ratio and LDL-C/HDL-C ratio as compared to normal control and treated groups. On treatment with standard and test extracts the cardiac risk is reduced. This reduction in cardiac risk is most profound in EEVA, HEVA and atorvastatin and even lower as compared to normal control and significantly lower as compared to disease control.

In the course of treating hyperlipidemia, most of the antihyperlipidemic drugs causes liver injury of hepatocellular pattern with very rare incidence of cholestatic damage. The most common mechanism

**Table 3**: Effects of *Vernonia anthelmintica* on fasting blood glucose in HFHS Rat model

| Treatment Group | Diet | Dose mg/kg | Blood glucose levels mg/dl |
|---|---|---|---|
| Control (NC) | Normal | Distilled water | 124±2.97 [a]** |
| Disease control (DC) | HFHS | Distilled water | 182.1±8.16c**, b** |
| Atorvastatin (SC) | HFHS | 2.1 | 126.6±7.7 a** |
| HEVA | HFHS | 300 | 119.0±4.29 a** |
| EEVA | HFHS | 300 | 116.3 ±10.99 a** |
| WDVA | HFHS | 300 | 139.6±8.52 a** |

**Table 4**: Effects of *Vernonia anthelmintica* on liver function in HFHS model

| Treatment Group | Diet | Dose mg/Kg | ALT U/L | GGT U/L | ALP U/L | AST U/L | TBR U/L |
|---|---|---|---|---|---|---|---|
| Control (NC) | Normal | Distilled water | 77.2± 4.08 | 1.17±0.29 | 288.4±9.36 | 134.5±5.8 a*, b** | 0.25±0.03 b* |
| Disease control (DC) | HFHS | Distilled water | 84.1± 4.23 | 1.57±0.20 | 352.0±28.5 b** | 166.4±8.6 c*, b** | 0.15±0.03 b** |
| Atorvastatin (SC) | HFHS | 2.1 | 114.0± 13.6 | 2.31± 0.32 | 247.3±15.1 a** | 202.1±7.0 c** | 0.67±0.13 a**, c* |
| HEVA | HFHS | 300 | 77.8± 4.95 | 1.95±0.36 | 296.4±17.1 | 149.7±6.9 b** | 0.87±0.10 a**,c* |
| EEVA | HFHS | 300 | 71.9± 4.95 | 1.25±0.43 | 273.3±17.7 | 161.0±5.3 b** | 0.45±0.14 |
| WDVA | HFHS | 300 | 71.2±72.34 | 2.58±0.50 | 252.4±32.7 | 190.3±4.0 c** | 0.35±0.01 |

n=8.
Values are expressed as mean ±S.E.M.
*P ≤ 0.05 significant; **P ≤0.005 highly significant
a different as compared to disease control
b different as compared to standard
c different as compared to control
HEVA= Hexane extract of *Vernonia anthelmintic.*
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proposed for these damages are alteration in cytochrome P450 system, disruption in bile acid functions, immune-mediated apoptosis and inflammatory responses to the drug or its metabolite and oxidative damage (Bertolami, 2005). Present study was thus designed to estimate the effects of *Vernonia anthelmintica* extracts on liver while treating hyperlipidemia in HFHS model.

AST (aspartate transaminase) and ALT (alanine transaminase) are predominantly concentrated in liver. However, AST is also present in skeletal muscles, heart, kidneys, blood and brain cells whereas ALT is also present in kidneys and skeletal muscles. Thus ALT is more specific for indicating hepatic damage. Elevation of both transaminases indicates the hepatocellular damage (Rej, 1989). ALP is an enzyme that is responsible for the transportation of metabolites though cell membranes. Elevation of ALP is the sign of liver and bone diseases as at these sites ALP is present predominantly (Dufour *et al*., 2000). ALP of hepatic origin is present on the surface of epithelium of bile duct. Cholestasis increases the production and release of ALP while bile salt accumulation also leads to its increase (Moss, 1997). Drug induced liver damage may appear as cholestatic pattern with preferential increase in ALP and conjugated bilirubin and negligible increase in amino transferases (Velayudham and Farrell, 2003). GGT (gamma glutamyl transpeptidase) is an important enzyme present in biliary epithelial cells, renal tubules, pancreas and intestine. Same mechanism of alteration as described for alkaline phosphatase is applicable here. Though it lacks specificity, it is very sensitive for liver disease and its alteration in context with changes in ALP is highly diagnostic for liver damage (Giannini *et al.*, 2005). In the present study, disease control of HFHS model revealed increased ALP levels which may be an indication of cholestatic pattern whereas ALT, AST, GGT and total bilirubin showed no significant alterations. Hence disease model showed no hepatocellular damage.

The HMG-CoA reductase inhibitors are the class of drugs used for the treatment of hyperlipidemia and prevention

of coronary artery disease. Atorvastatin is reported to be associated with hepatic injury (Liu *et al*., 2010). Present study has evaluated its effects on liver in HFHS model of hyperlipidemia. The results revealed that atorvastatin has increased AST as compared to control whereas ALT and GGT were not significantly different as compared to control and disease control.

Total bilirubin levels were also increased as compared to control and disease control. However, ALP levels were found to be decreased as compared to disease control that revealed the improvement of cholestatic pattern seen in disease control. Thus atorvastatin showed mild potential of causing hepatocellular damage but improved the cholestatic damage. EEVA and HEVA showed most favorable effects on liver enzymes with no marked alterations in liver enzymes as compared to control. It is worth mentioning that HEVA and EEVA showed significantly lower levels of AST as compared to atorvastatin. Total bilirubin levels showed no marked elevation except for HEVA that revealed significantly higher bilirubin levels as compared to control and disease control. As the ALP levels showed no significant increase, it would not indicate cholestatic damage. WDVA showed significantly increased levels of AST and thus showed a hepatocellular damage potential.

The results of the present study revealed the safety of HEVA and EEVA in the course of treating hyperlipidemia in rats. This safety may be attributed to antioxidant activity of these extracts.

Increased intake of sugar and high-fat diet is associated with insulin resistance and if untreated, resulted in diabetes. Lipogenic effects of high sugars involves increased triglyceride synthesis in liver after reaching to hepatocytes though GLUT-5 i.e. insulin-independent glucose transporter (Armato *et al*., 2015). Hypertriglyceridemia might be the reason for insulin resistance characterized by impaired carbohydrate metabolism, high LDL-C and VLDL-C and low HDL-C levels (Hsieh *et al*., 2013).

Present study revealed the same spectra of disorders in HFHS model. All these disturbances in carbohydrate and lipid metabolism were observed to be improved by treating with extracts of *Vernonia anthelmintica*. The results revealed significantly increased levels of fasting blood glucose in disease control as compared to normal control. The treatment with atorvastatin, EEVA, HEVA and WDVA for 30 days has resulted in significant decrease in fasting blood sugar. This decrease in FBS is most profound in EEVA followed by HEVA, atorvastatin and WDVA.

## CONCLUSION

*Vernonia anthelmintica* seed extracts and water decoction have shown promising hypolipidemic and hypoglycaemic effects with favourable hepatic profile, however, WDVA should be further investigated for its effects on liver. Additional studies should be designed to strengthen these findings on histopathological and mechanistic grounds.

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